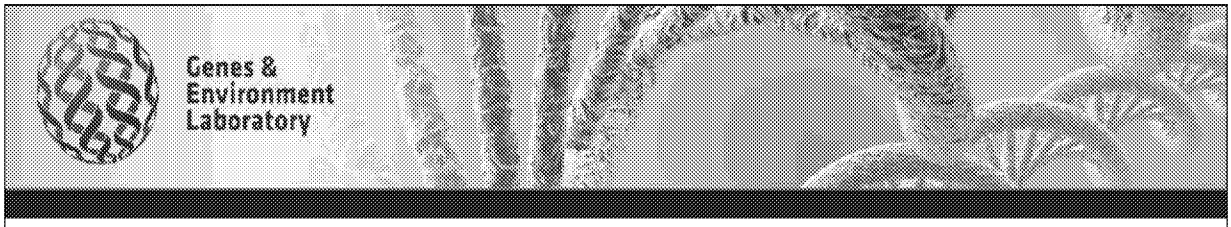

Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis

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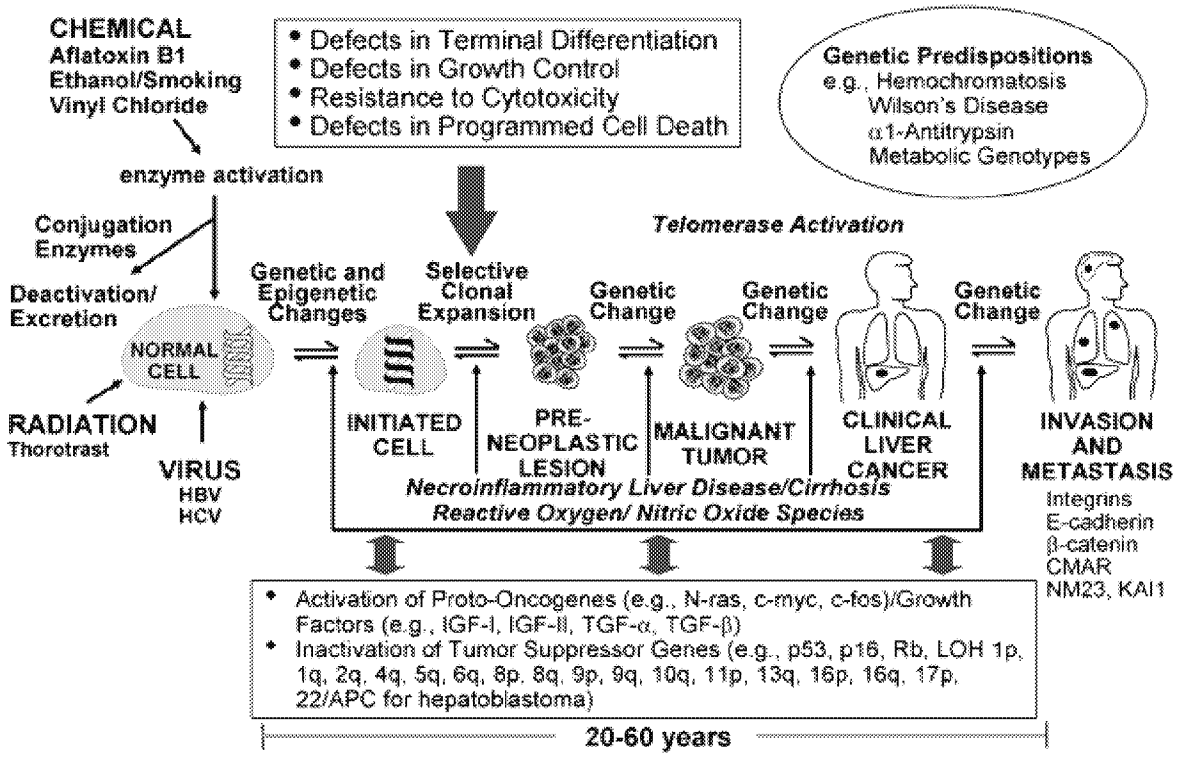
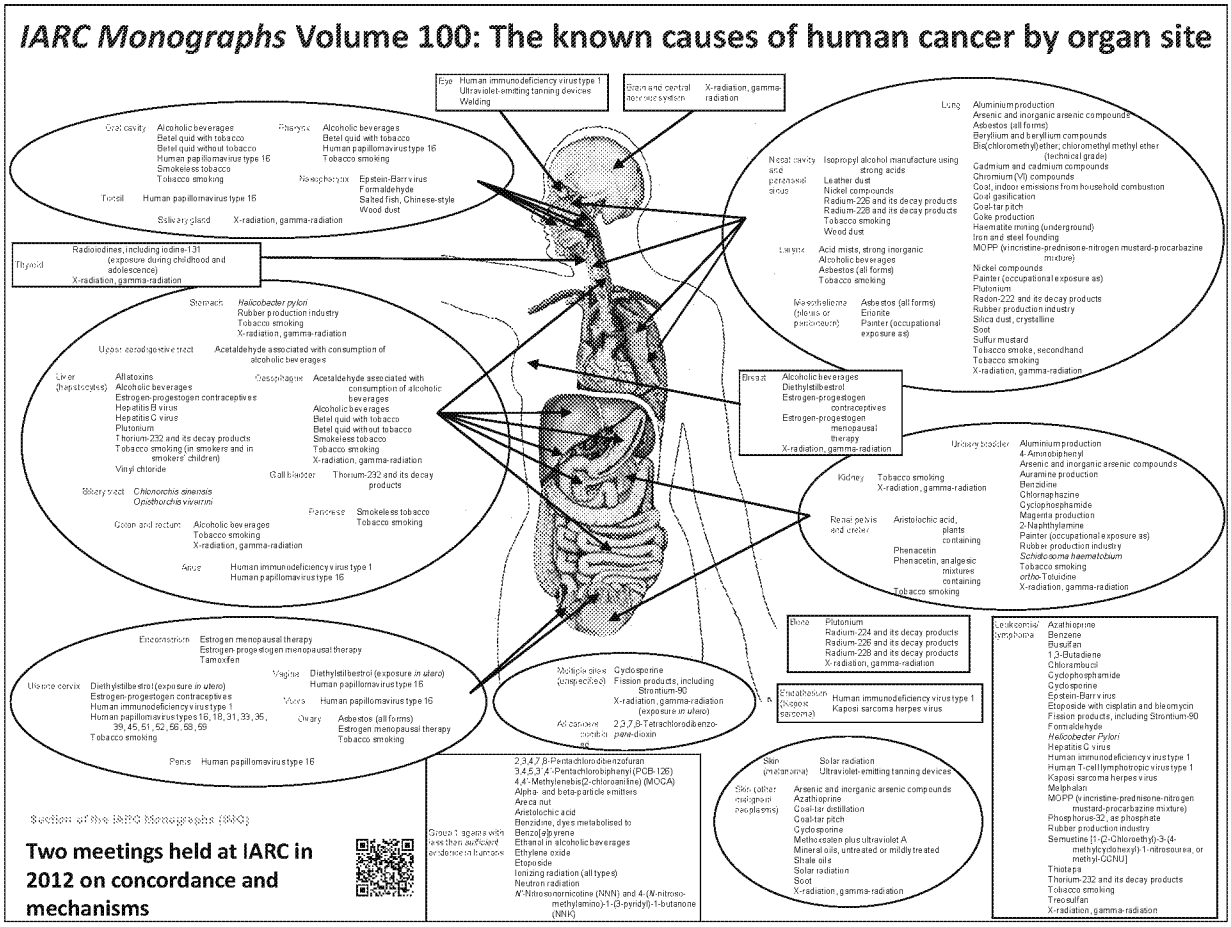
Mechanistic data - Problems to address

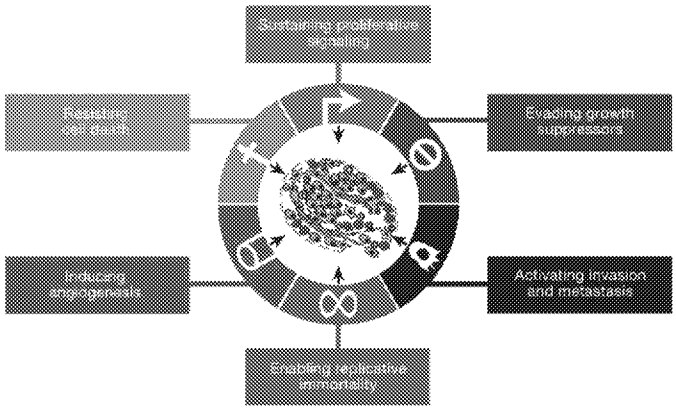
- There is no broadly accepted, systematic method for identifying, organizing, and summarizing mechanistic data for the purpose of decision-making in cancer hazard identification
- Many human carcinogens act via multiple mechanisms causing various biological changes in the multistage process of carcinogenesis – How to capture these diverse effects that lead to cancer and other adverse outcomes for all types of agents?

Human Tumors and Stages of Carcinogenesis



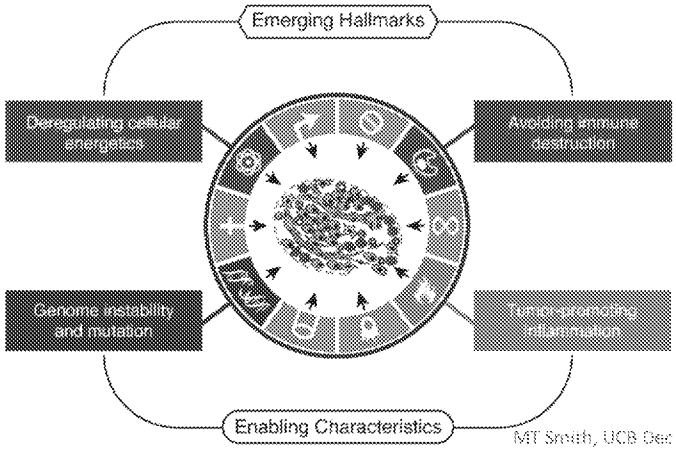
Hussain *et al.*, Oncogene, 2007





HALLMARKS OF CANCER

1. Sustaining proliferative signaling
2. Evading growth suppressors
3. Resisting cell death
4. Enabling replicative immortality
5. Inducing aberrant angiogenesis
6. Activating invasion & metastasis



Emerging Hallmarks

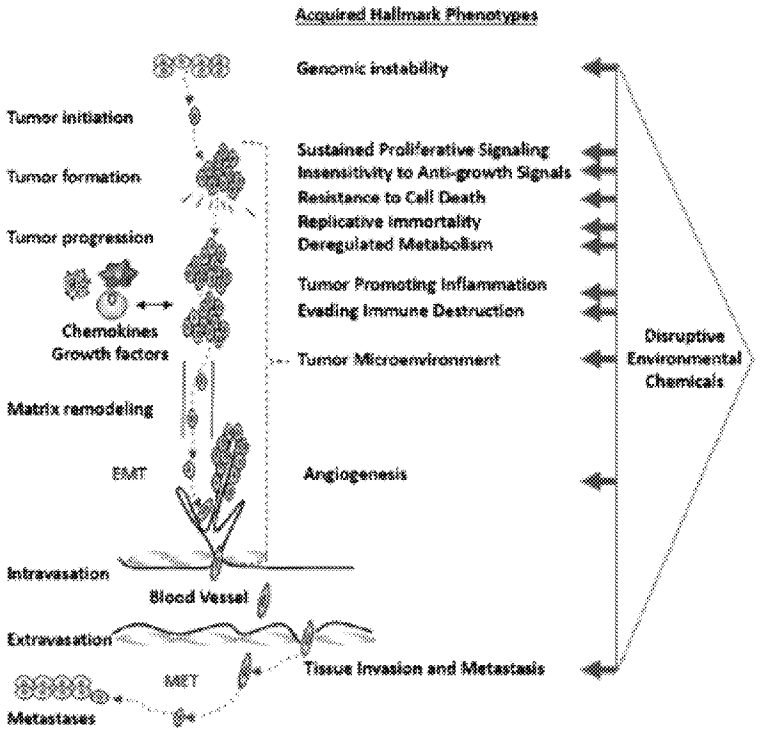
- Reprogramming energy metabolism
- Evading immune destruction

Enabling Characteristics

- Genomic instability and mutation
- Inflammation

Hanahan and Weinberg 2011

Chemicals and other stressors act at different points on the disease continuum



"Considering the multistep nature of cancer and the acquired capabilities implied by each of these hallmarks, it is therefore a very small step to envision how a series of complementary exposures acting in concert might prove to be far more carcinogenic than predictions related to any single exposure might suggest. Interacting contributors need not act simultaneously or continuously, they might act sequentially..."

Goodson et al. Carcinogenesis. 2015 Jun; 36(Suppl 1): S254–S296.

Review team	Chemical name	Disruptive action on key mechanism/pathway	Low-dose effect (LDE, LLDE, NLDE, threshold, unknown)
Angiogenesis	Diniconazole	Vascular cell adhesion molecule and cytokine signaling	Threshold (H-PC) (36 =TOXCAST)
	Chlorothalonil	Thrombomodulin, vascular proliferation and cytokine signaling	Unknown (H-PC) (36), NLDE (A-*in vivo*) (38 in Amphibians)
Immune system evasion	Pyridaben	Chemokine signaling, TGF-β, FAK, HIF-1a, IL-1a pathways	Unknown (H-CL, H-PC, A-CL) (36,139,140), threshold (A-I) (141)
	Triclosan	Chemokine signaling, TGF-β, FAK, IL-1a pathways	Threshold (H-CL, H-PC, A-I) (36,142–144), LDE (A-I, H-CL) (145,146) None of these papers (142-146) show immune evasion

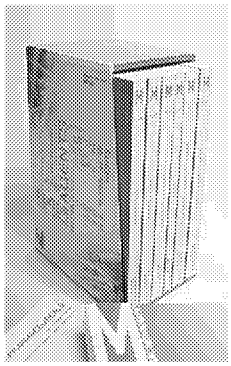
Examples of endpoints used to support conclusions of Goodson et al. --

MT Smith, UCB Dec 2015

Problem is that assay endpoints don't match hallmarks

Dilemma: Cancer or Carcinogens

- Hallmarks are the biological characteristics of cancer cells and tumors in general, NOT the characteristic properties of human carcinogens
- Need to identify the key characteristics of human carcinogens
- IARC Working Group did this in 2012 and subsequently scientists at EPA, IARC and elsewhere determined how these characteristics could be searched for systematically



Multiple Mechanisms of IARC Group 1 Carcinogens

[KZ Guyton….MT Smith, Mut Res 681; 230, 2009]

	Carcinogen			
Mechanisms	AFB1	As+3	Asbestos	Benzene
DNA damage	+	+	-	+
Gene mutation	+	-	+	-
Chrom mutation	+	+	+	+
Aneuploidy	-	+	+	+
Epigenetic	+	+		+
Receptor signaling	-	+	+	
Other signaling	-	+		+
Immune effects	+	+	+	+
Inflammation	+	+	+	+
Cytotoxicity	+	+	+	+
Mitogenic	-	+		-
Gap junction	+	+		+

Key Characteristics of Human Carcinogens

Key characteristic:
1. Is Electrophilic or can be metabolically activated
2. Is Genotoxic
3. Alters DNA repair or causes genomic instability
4. Induces Epigenetic Alterations
5. Induces Oxidative Stress
6. Induces chronic inflammation
7. Is Immunosuppressive
8. Modulates receptor-mediated effects
9. Causes Immortalization
10. Alters cell proliferation, cell death, or nutrient supply

Evidence that these characteristics are observed, especially in humans or as intermediate biomarkers in human specimens can provide biological plausibility for epidemiological findings and/or early warning if no epidemiology exists

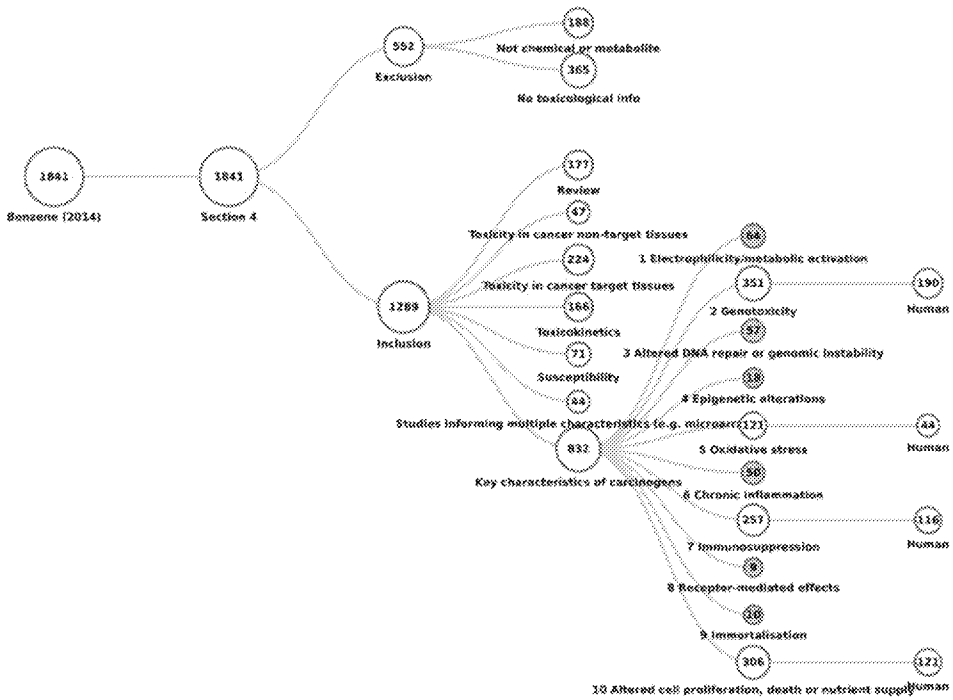
Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert P, Hecht SS, Bucher JR, Stewart BW, Baan R, Cogliano VJ and K Straif. *Env Health Persp., in press*, http://ehp.niehs.nih.gov/15-09912/

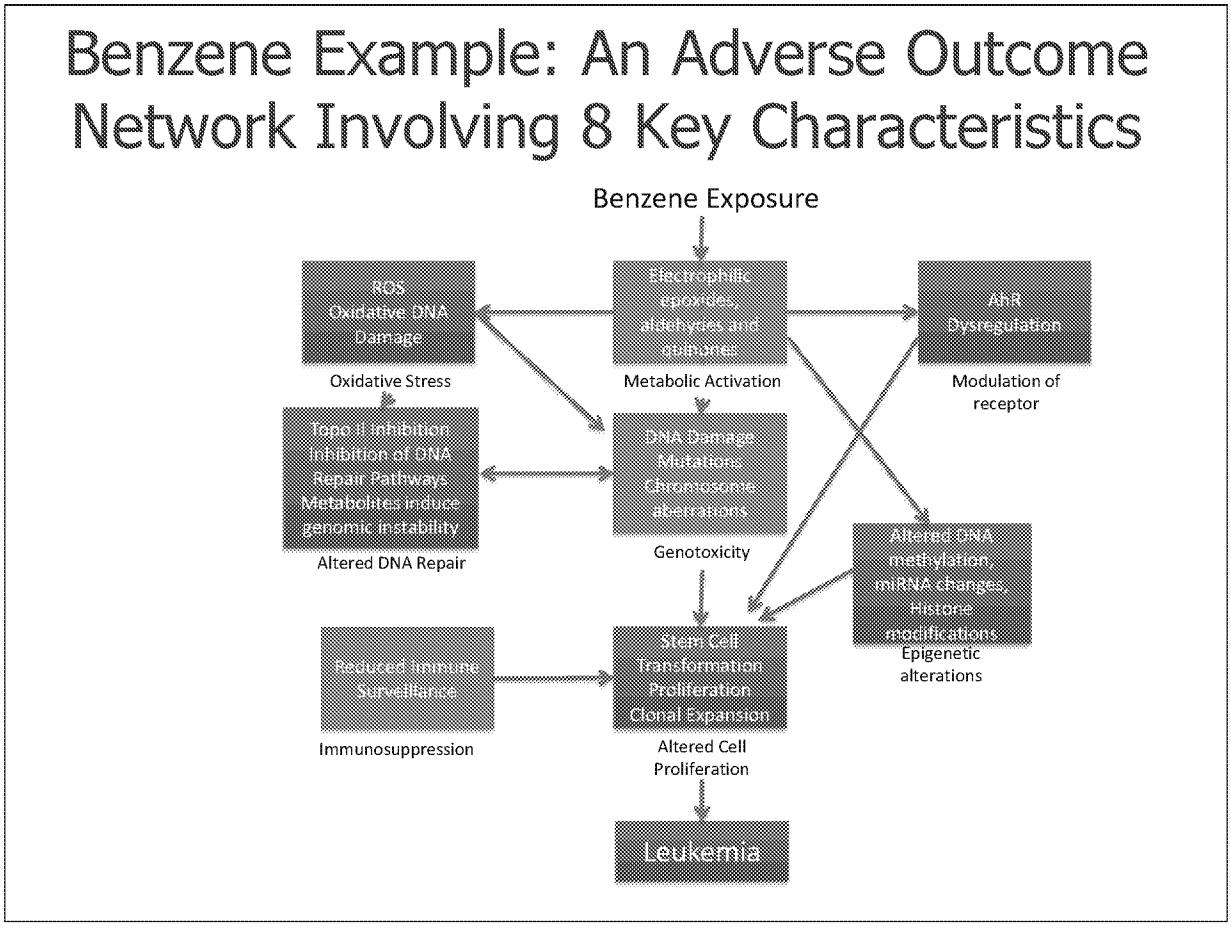
Characteristic	Examples of relevant evidence
1. Is Electrophilic or Can Be Metabolically Activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.
2. Is Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces Epigenetic Alterations	DNA methylation, histone modification, microRNA expression
5. Induces Oxidative Stress	Oxygen radicals, oxidative damage to macromolecules (e.g., DNA, lipids)

Characteristic	Examples of relevant evidence
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation, altered telomeres
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Benzene Mechanistic Data Search

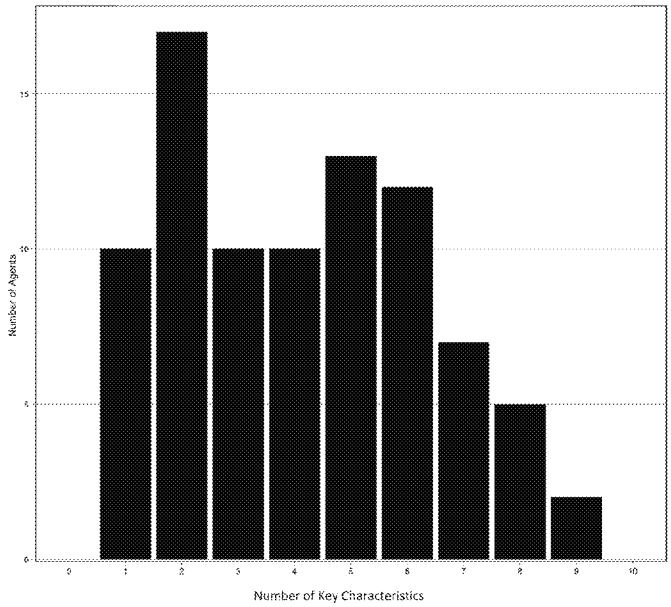
conducted using the Health Assessment Workplace Collaborative (HAWC) Literature Search tool (https://hawcproject.org/)





An overview of how benzene induces 8 of the key characteristics in a probable mechanism of carcinogenicity. A full review of these mechanistic data is given in (McHale et al. 2012), from which this Figure was adapted

Number of IARC Group-1 Agents Demonstrating Multiple Key Characteristics



D. Krewski et al. in Monograph from IARC Working Group on 'Tumour-site Concordance and Mechanisms of Carcinogenesis', in press.

Implications of 'key characteristics'

- Lays the groundwork for a structured evaluation of the strength of the mechanistic evidence base, and therefore its utility in supporting hazard classifications.
- Shows carcinogens tend to act through multiple mechanisms – separation into genotoxic and non-genotoxic actions of little value
- Allows development of credible Adverse Outcome Networks based on systematic review
- Could be developed for specific cancers and other adverse outcomes
- HT assays need to be developed based on characteristics and hallmarks. Current ones flawed.

An Agency-Academia Collaboration

- **IARC**: Kathryn Z. Guyton, Robert Baan and Kurt Straif
- **US EPA**: Catherine F. Gibbons, Jason M. Fritz, David M. DeMarini, Jane C. Caldwell, Robert Kavlock, Vincent Cogliano
- **NTP**: John R. Bucher
- **Academia**: Ivan Rusyn, Paul Lambert, Stephen S. Hecht, Bernard W. Stewart
- **Thun**: Christopher Portier
- **Other members** of the IARC WG: Lawrence Banks; Frederick A. Beland,; James A. Bond; Maarten C. Bosland; Bice Fubini; Bernard D. Goldstein; Kari Hemminki; Mark A. Hill; Charles Jameson; Agnes B. Kane; Daniel Krewski; Ronald Melnick; Jerry M. Rice; Leslie Stayner; Robert L. Ullrich; Harri Vainio; Paolo Vineis; Michael P. Waalkes; and, Lauren Zeise.
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